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Gene Engineering Experiment Analysis Utilizing Synthesize d Strains Instead Of Living Sars-Cov-2 Strains

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Abstract: With genome conformation capture (Gene engineering), we have shown that the linear organization of the genome is a true 3D structure that turns neighboring genes into plasmids, which is undoubtedly the most powerful tool in the toolbox of molecular biology that can be used for future microbes that could be the solution to many of the problems that humanity will face in the future. The genomics method has shown that the adjacent genes become small factories that are co-regulated and co-expressed through genome conformation capture, showing that protein-protein interactions form the basis of many different protein types and their interaction with other proteins. It was only in the 1970s that researchers began to manipulate DNA by using gene engineering and developing the first technology for sequencing human genomes. The era of gene engineering and technology began in the late 1960s with the discovery of the “DNA ligase” and its use as a tool for genome engineering.

1. Introduction

Gene editing or gene engineering is when one alters the genome manually to have the desired effect on a certain organism. CRISPR, which stands for clustered regularly interspaced short palindromic repeats, is a cheap, fast, and accurate way to accomplish gene editing (Broad institute 2018).

2. Literature Review

2.1 Crispr

The technology utilizes the self-defense system of various bacteria in nature (Robert Sanders & Sanders, 2018). In such bacteria, there is a string of DNA that contains palindromic repeats, spacers, and Cas DNA. During a viral invasion, the DNA activates, resulting in Cas protein complexes, a nuclease, and crRNA from spacers, identifying and cutting the viral genome. If the foreign gene had not been encountered before, i.e. unrecognizable by the crRNA, a special Cas protein will eliminate the gene and bring back a section of the virus, which in turn becomes a new spacer.

2.2 Crispr-Cas System

The most prominent discovery in the CRISPR-Cas system is Cas9, a DNA nuclease that gained CRISPR its fame. Cas9 functions similar to the majority of Cas proteins where it, combined with trans-activating crRNA (tracrRNA) and precursor crRNA (pre-crRNA), targets a certain DNA sequence encoded on the spacer and slices it apart (Cong et al., 2013). However, Jennifer Doudna and Emmanuelle Charpentier first proposed in 2012 about their experiment of synthesizing a guideRNA (gRNA) which allows one to target a DNA sequence as one wishes (Zhu, 2017). Being a combination of a tracrRNA and a crRNA, a manually designed gRNA can locate the target DNA sequence and guide the Cas9 protein into slicing it (Zhu, 2017). Cas13, on the other hand, has the ability to cut RNA instead of DNA (Abudayyeh et al., 2017). Moreover, Cas13's RNase acts independently from other RNase while having an exposed catalytic site, making it non-specific when activated (Abudayyeh et al., 2017).

2.3 Sherlock

An example of utilization of Cas13 is SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) in its detection of SARS-CoV-2. A gRNA was designed to target the virus, and if the virus is indeed present, the Cas13 protein will be activated, which in turn, slicing the surrounding RNA sequences, including the reporters (McGovern Institute 2020). Thus, from sensing the presence of sliced reporters, one can tell whether SARS-CoV-2 is present in the sample (McGovern Institute 2020).

2.4 Pac-Man and What It Had Accomplished

Although SHERLOCK is able to detect SARS-CoV-2, a Stanford team with their PAC-MAN (prophylactic antiviral CRISPR in human cells) can directly fight COVID (Abbott et al., 2020). The team chose the class 2 type VI-D CRISPR-Cas13d system (from Ruminococcus flavefaciens XPD3002) for its "relatively small size", "high specificity", and "strong catalytic activity" (Abbott et al., 2020).The team had tested PAC-MAN on an H1N1 strain of IAV (influenza A virus), which they claim has "a similar tropism as SARS-CoV-2 for respiratory tract epithelial cells" (Abbott et al., 2020). Sample cells were transfected with crRNA and two days later tested with PR8-mNeon, a mNeon gene expressing strain of H1N1, and a fluorescent reporter protein (multiplicity of infection was at 2.5 or 5.0 stating such high MOI is needed to appropriately detect the infection condition) (Abbott et al., 2020). With their customized spacer in the crRNA, the team was able to target 91% of the virus with 6 crRNA groups and 100% with 22 groups (Abbott et al., 2020).

3. Problems Statement

The experiment is a proof-of-concept while utilizing synthesized strains instead of living SARS-CoV-2 strains (Abbott et al., 2020), one is unable to be certain if PAC-MAN can be consistent facing the real virus. In addition, since the virus mainly targets the patient's lungs, it is unknown how the team can transfer PAC-MAN to the infected area (Abbott et al., 2020). All CRISPR Cas proteins can only have an effect on the direct cell (Abudayyeh et al., 2017), the percentage of the epithelial cells which should be under the effect in order to truly inhibit the growth and spread of the virus in the patient is unknown. Moreover, since Cas13 also targets other RNA sequences in the cell as it activates, the side

effects and damage PAC-MAN can bring to the patient while combating COVID is also under question (Abudayyeh et al., 2017).

4. Findings

The main concern is that Cas13, as it activates, destroys all the RNA of a cell, which in turn will at least disable many of its crucial functions. However, since COVID-19 replicates in "astronomical numbers" (Lambert, 2020), so if PAC-MAN was able to be successfully delivered to the infected area and destroyed all the viruses, one may need to consider the fact that if the patient is able to survive with the remaining cells. Thus, one can propose that PAC-MAN should be allocated preemptively so it can eliminate the virus before it replicates, significantly reducing the casualties of the local cells.

However, if one wishes to not use PAC-MAN like a vaccine but to treat patients with an ongoing RNA viral infection, SARS-CoV-2 with its explosive replication is not a good target for one to use PAC-MAN. As the gRNA can be engineered, PAC-MAN has the potential to combat future viral diseases, yet it is suggested that the technology should combat infections in an early stage and the virus is relatively slow to spread, while the cells of the infected area should be regeneratable and not crucial for the patient to survive.

Under the assumption that different gRNA can be synthesized to target other RNA viruses, PAC-MAN has the potential to combat other RNA viruses. Hereby lists some well-known ones: Influenza, Ebola, HIV, Polio, Measles. The genome of influenza viruses is a single strained, negative sense RNA (WHO, 2020). Due to its characteristics of targeting the patient's epithelial cells of nose/pharynx/trachea/bronchi (Oxford & Hockley, 2008), influenza A virus is sometimes mentioned when talking about COVID. Therefore, one can argue that the tactics deployed against SARS-CoV-2 can also be utilized against Influenza (mainly type A). RNA viruses like Ebola and HIV would infect one's immune cells (Harvard University, 2014;

Fanales-Belasio, Raimondo, Suligoi, & Buttò, 2010), therefore, PAC-MAN is not the best solution against such viruses since a damaged immune system after the activation of Cas13 might put the patient under danger from other problems. Measles is a very contagious, single stranded, enveloped RNA virus (CDC, 2018). Like many other viruses that enter the body through respiratory tracks, it targets the patient's lungs, in this case, the macrophages and dendritic cells (Shultz, 2015). However, measles would then spread to the lymph nodes, and eventually to the B and T cells of the human body (Shultz, 2015). Thus, PAC-MAN is better to be used to deal with early measle infections or only in the lungs, further intervention with infections on the lymph nodes can again affect one's immune systems.

5. Conclusion

Poliovirus, the "prototype member of the Picornaviridae family", first tackles the epithelial cells of the intestines with its surface protein (Belov et al., 2011). In this early stage, one can say that PAC-MAN can be utilized for combating the virus. Yet it will then spread through the bloodstream to the entire body, infecting the motor neurons (Belov et al., 2011), meaning that PAC-MAN is not recommended for this stage as it may harm the cells along with the virus.

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